Todar's Online Textbook of Bacteriology

MECHANISMS OF BACTERIAL PATHOGENICITY: COLONIZATION AND INVASION

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Introduction

Microbial **pathogenicity** has been defined as the structural and biochemical mechanisms whereby microorganisms cause disease. Pathogenicity in bacteria may be associated with unique structural components of the cells (e.g. capsules, fimbriae, LPS or other cell wall components) or active secretion of substances that either damage host tissues or protect the bacteria against host defenses. Hence, there are two broad qualities of pathogenic bacteria that underlie the means by which they cause disease: **invasiveness and toxigenesis.**

Toxigenesis is the ability to produce toxins. Toxic substances produced by bacteria, both soluble and cell-associated, may be transported by blood and lymph and cause cytotoxic effects at tissue sites remote from the original point of invasion or growth.

Invasiveness is the ability of a pathogen to invade tissues. Invasiveness encompasses (1) mechanisms for colonization (adherence and initial multiplication), (2) production of extracellular substances ("invasins"), that promote the immediate invasion of tissues and (3) ability to bypass or overcome host defense mechanisms which facilitate the actual invasive process. This chapter deals with the first two aspects of of invasiveness: colonization and invasion.

COLONIZATION

The first stage of microbial infection is **colonization**: the establishment of the pathogen at the appropriate portal of entry. Pathogens usually colonize host tissues that are in contact with the external environment. Sites of entry in human hosts include the urogenital tract, the digestive tract, the respiratory tract and the conjunctiva. Organisms that infect these regions have usually developed tissue adherence mechanisms and some ability to overcome or withstand the constant pressure of the host defenses at the surface.

Bacterial Adherence to Mucosal Surfaces. In its simplest form, bacterial adherence or attachment to a eucaryotic cell or tissue surface requires the participation of two factors: a **receptor** and a **ligand**. The receptors so far defined are usually specific carbohydrate or peptide residues on the eucaryotic cell surface. The bacterial ligand, called an **adhesin**, is typically a macromolecular component of the bacterial cell surface which interacts with the host cell receptor. Adhesins and receptors usually interact in a complementary and specific fashion with specificity comparable to enzyme-substrate relationships and antigen-antibody reactions. Table 1 is a list of terms that are used in medical microbiology to refer to microbial adherence to surfaces or tissues.

TABLE 1. TERMS USED TO DESCRIBE ADHERENCE FACTORS IN MICROBIOLOGY

ADHERENCE FACTOR	DESCRIPTION
Adhesin	A surface structure or macromolecule that binds a bacterium to a specific surface
Receptor	A complementary macromolecular binding site on a (eucaryotic) surface that binds specific adhesins or ligands
Lectin	Any protein that binds to a carbohydrate
Ligand	A surface molecule that exhibits specific binding to a receptor molecule on another surface
Mucous	The mucopolysaccharide layer of glucosaminoglycans covering animal cell mucosal surfaces
Fimbriae	Filamentous proteins on the surface of bacterial cells that may behave as adhesins for specific adherence
Common pili	Same as fimbriae
Sex pilus	A specialized pilus that binds mating procaryotes together for the purpose of DNA transfer
Type 1 fimbriae	Fimbriae in *Enterobacteriaceae* which bind specifically to mannose terminated glycoproteins on eucaryotic cell surfaces
Type 4 pili	Pili in certain Gram-positive and Gram-negative bacteria. In *Pseudomonas*, thought to play a role in adherence and biofilm formation
Biofilm	exopolysaccharide or slime produced by bacteria that attaches imbedded cells to a surface
S-layer	Proteins that form the outermost cell envelope component of a broad spectrum of bacteria, enabling them to adhere to host cell membranes and environmental surfaces in order to colonize.
Glycocalyx	A layer of exopolysaccharide fibers on the surface of bacterial cells which may be involved in adherence to a surface. Sometimes a general term for a bacterial capsules.
Capsule	A detectable layer of polysaccharide (rarely polypeptide) on the surface of a bacterial cell which may mediate specific or nonspecific attachment
Lipopolysaccharide (LPS)	A distinct cell wall component of the outer membrane of Gram-negative bacteria with the potential structural diversity to mediate specific adherence. Probably functions as an adhesin
Teichoic acids and lipoteichoic acids (LTA)	Cell wall components of Gram-positive bacteria that may be involved in nonspecific or specific adherence

Specific Adherence of Bacteria to Cell and Tissue Surfaces

Several types of observations have provided indirect evidence for **specificity of adherence** of bacteria to host cells or tissues:

1. **Tissue tropism.** Particular bacteria are known to have an apparent preference for certain tissues over others, e.g. *S. mutans* is abundant in dental plaque but does not occur on epithelial surfaces of the tongue; the reverse is true for *S. salivarius* which is attached in high numbers to epithelial cells of the tongue but is absent in dental plaque. *Corynebacterium diphtheriae* colonizes exclusively in the throat.

2. Species specificity. Certain pathogenic bacteria infect only certain species of animals, e.g. *N. gonorrhoeae* and *Bordetella pertussis* infections are limited to humans; enteropathogenic *E. coli* K-88 infections are limited to pigs; *E. coli* CFA I and CFA II infect humans; *E. coli* K-99 strains infect calves.; Group A streptococcal infections occur only in humans. In addition, certain indigenous species and symbionts are quite specific in their associations with specific animal hosts.

3. Genetic specificity within a species: certain strains or races within a species may be genetically immune to a pathogen, e.g. certain pigs are not susceptible to *E. coli* K-88 infections; males are not susceptible to mastitis; females are not susceptible to orchitis; A percentage of females are not susceptible to urinary tract infection (UTI) caused by *E. coli*.

Although other explanations are possible, the above observations might be explained by the existence of specific interactions between microorganisms and eucaryotic tissue surfaces which allow microorganisms to become established on the surface.

Mechanisms of Adherence to Cell or Tissue Surfaces

The mechanisms for adherence may involve two steps:

1. nonspecific adherence: reversible attachment of the bacterium to the eucaryotic surface (sometimes called "docking")

2. specific adherence; irreversible permanent attachment of the microorganism to the surface (sometimes called "anchoring").

The usual situation is that reversible attachment precedes irreversible attachment but in some cases, the opposite situation occurs or specific adherence may never occur.

Nonspecific adherence involves nonspecific attractive forces which allow approach of the bacterium to the eucaryotic cell surface. Possible interactions and forces involved are:

1. hydrophobic interactions

2. electrostatic attractions

3. atomic and molecular vibrations resulting from fluctuating dipoles of similar frequencies

4. Brownian movement

5. recruitment and trapping by biofilm polymers interacting with the bacterial glycocalyx (capsule)

Specific adherence involves permanent formation of many specific lock-and-key bonds between complementary molecules on each cell surface. Complementary receptor and adhesin molecules must be accessible and arranged in such a way that many bonds form over the area of contact between the two cells. Once the bonds are formed, attachment under physiological conditions becomes virtually irreversible.
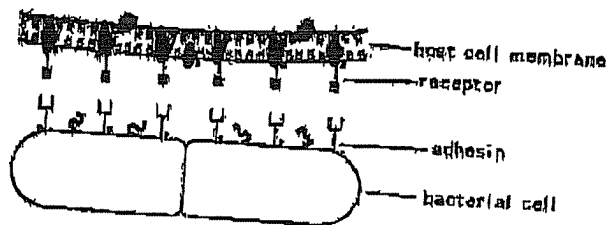
Specific adherence involves complementary chemical interactions between the host cell or tissue surface and the bacterial surface. In the language of medical microbiologist, a bacterial "adhesin" attaches covalently to a host "receptor" so that the bacterium "docks" itself on the host surface. The adhesins of bacterial cells are chemical components of capsules, cell walls, pili or fimbriae. The host receptors are usually glycoproteins located on the cell membrane or tissue surface.

Several types of experiments provide direct evidence that receptor and/or adhesin molecules mediate specificity of adherence of bacteria to host cells or tissues. These include:

1. The bacteria will bind isolated receptors or receptor analogs.

2. The isolated adhesins or adhesin analogs will bind to the eucaryotic cell surface.

3. Adhesion (of the bacterium to the eucaryotic cell surface) is inhibited by:

    a. isolated adhesin or receptor molecules

    b. adhesin or receptor analogs

    c. enzymes and chemicals that specifically destroy adhesins or receptors

   d. antibodies specific to surface components (i.e., adhesins or receptors)

Some Specific Bacterial Adhesins and their Receptors

The adhesins of *E. coli* are their common pili or fimbriae. A single strain of *E. coli* is known to be able to express several distinct types of fimbriae encoded by distinct regions of the chromosome or plasmids. This genetic diversity permits an organism to adapt to its changing environment and exploit new opportunities presented by different host surfaces. Many of the adhesive fimbriae of *E. coli* have probably evolved from fimbrial ancestors resembling Type-I and Type IV pili.

Type-I fimbriae enable *E. coli* to bind to D-mannose residues on eucaryotic cell surfaces. Type-I fimbriae are said to be "mannose-sensitive" since exogenous mannose blocks binding to receptors on red blood cells. Although the primary 17kDa fimbrial subunit is the major protein component of Type-1 fimbriae, the mannose-binding site is not located here, but resides in a minor protein (28-31kDa) located at the tips or inserted along the length of the fimbriae. By genetically varying the minor "tip protein" adhesin, the organisms can gain ability to adhere to different receptors. For example, tip proteins on pyelonephritis-associated (pap) pili recognize a galactose-galactose disaccharide, while tip proteins on S-fimbriae recognize sialic acid. S fimbriae are able to recognize receptor molecules containing sialic acid and are produced by pathogenic *E. coli* strains causing urinary tract infection.

Pseudomonas, *Vibrio* and *Neisseria* possess Type IV pili that contain a protein subunit with a methylated amino acid, often phenylalanine, at or near its amino terminus. These "N-methylphenylalanine pili" have been established as virulence determinants in pathogenesis of

Pseudomonas aeruginosa lung infection in cystic fibrosis patients. These type of fimbriae occur in *Neisseria gonorrhoeae* and their receptor is thought to be an oligosaccharide. Type IV pili are the top (toxin coregulated pili) fimbriae used in attachment of *Vibrio cholerae* to the gastrointestinal epithelium.
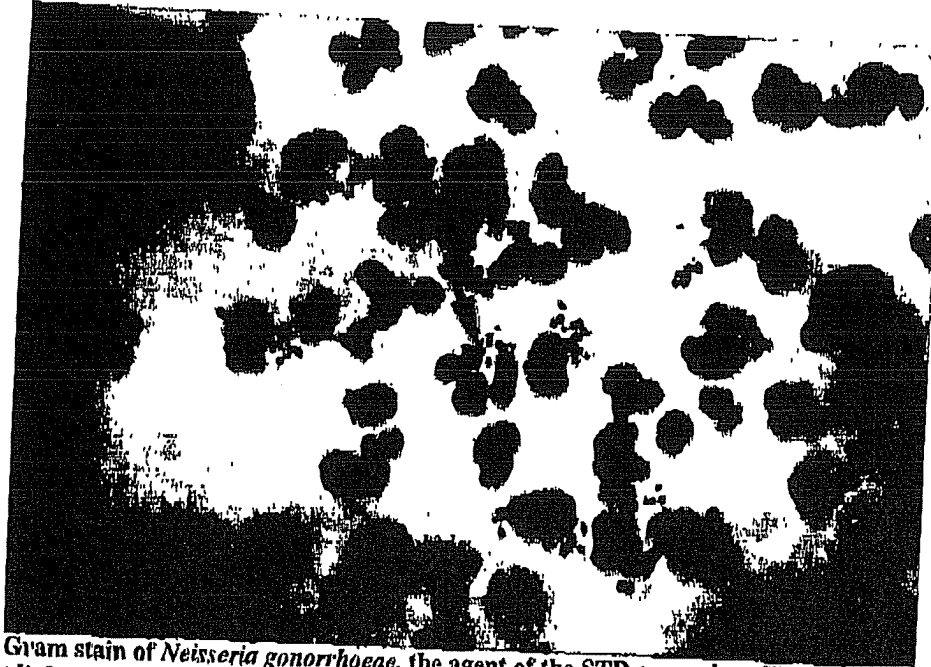
Gram stain of *Neisseria gonorrhoeae*, the agent of the STD gonorrhea. The bacteria are seen as pairs of cocci (diplococci) in association with host pmn's (polymorphonuclear leukocytes). Gonorrhea is the second most prevalent bacterial STD in the U.S. behind chlamydia. The bacterium has multiple determinants of virulence including the ability to attach to and enter host cells, resist phagocytic killing and produce endotoxins which eventually lead to an intense inflammatory response. CDC.
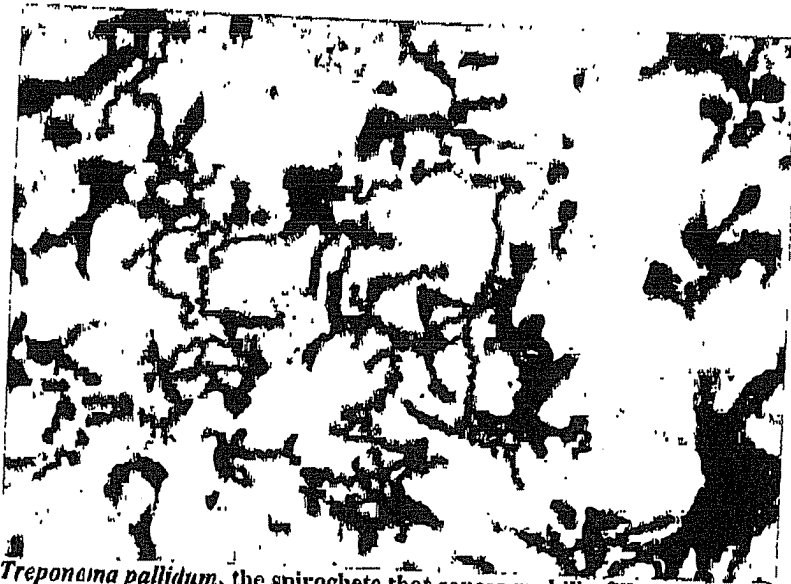
The adhesins of *Streptococcus pyogenes* are controversial. In 1972, Gibbons and his colleagues demonstrated that attachment of streptococci to the oral mucosa of mice is dependent on M protein. Ofek and Beachey argued that lipoteichoic acid (LTA), rather than M protein, was responsible for streptococcal adherence to buccal epithelial cells. In 1996, Hasty and Courtney proposed a two-step model of attachment that involved both M protein and teichoic acids. They suggested that LTA loosely tethers streptococci to epithelial cells, and then M protein secures a firmer, irreversible association. In 1992, protein F was discovered and found to be a fibronectin binding protein. More recently, in 1998, M proteins M1 and M3 were also found to bind to fibronectin. Apparently, *S. pyogenes* produces multiple adhesins with varied specificities.

Electron micrograph of *Streptococcus pyogenes* (Group A streptococci) by Maria Fazio and Vincent A. Fischetti, Ph.D. with permission. The Laboratory of Bacterial Pathogenesis and Immunology, Rockefeller University. The cell surface fibrils, that consist primarily of M protein, are clearly evident. The M protein has several possible roles in virulence: it is involved in adherence, resistance to phagocytosis, and in antigenic variation of the pathogen.

Staphylococcus aureus also binds to the amino terminus of fibronectin by means of a fibronectin-binding protein which occurs on the bacterial surface. Apparently, *S. aureus* and Group A streptococci use different mechanisms but adhere to the same receptor on epithelial surfaces.

Treponema pallidum has three related surface adhesins (P1, P2 and P3), which bind to a four-amino acid sequence (Arg-Gly-Asp-Ser) of the cell-binding domain of fibronectin. It is not clear if *T. pallidum* uses fibronectin to attach to host surfaces or coats itself with fibronectin to avoid host defenses (phagocytes and immune responses).

Treponema pallidum, the spirochete that causes syphilis. Silver stain, CDC.

TABLE 2. EXAMPLES OF SPECIFIC ATTACHMENTS OF BACTERIA TO HOST CELL OR TISSUE SURFACES

Bacterium	Adhesin	Receptor	Attachment site	Disease
Streptococcus pyogenes	Protein F	Amino terminus of fibronectin	Pharyngeal epithelium	Sore throat
Streptococcus mutans	Glycosyl transferase	Salivary glycoprotein	Pellicle of tooth	Dental caries
Streptococcus salivarius	Lipoteichoic acid	Unknown	Buccal epithelium of tongue	None
Streptococcus pneumoniae	Cell-bound protein	N-acetylhexosamine-galactose disaccharide	Mucosal epithelium	pneumonia
Staphylococcus aureus	Cell-bound protein	Amino terminus of fibronectin	Mucosal epithelium	Various
Neisseria gonorrhoeae	Type IV pili (N-methylphenyl-alanine pili)	Glucosamine-galactose carbohydrate	Urethral/cervical epithelium	Gonorrhea
Enterotoxigenic E. coli	Type-I fimbriae	Species-specific carbohydrate(s)	Intestinal epithelium	Diarrhea
Uropathogenic		Complex	Urethral	

E. coli	Type I fimbriae	carbohydrate	epithelium	Urethritis
Uropathogenic E. coli	P-pili (pap)	Globobiose linked to ceramide lipid	Upper urinary tract	Pyelonephritis
Bordetella pertussis	Fimbriae ("filamentous hemagglutinin")	Galactose on sulfated glycolipids	Respiratory epithelium	Whooping cough
Vibrio cholerae	N-methylphenylalanine pili	Fucose and mannose carbohydrate	Intestinal epithelium	Cholera
Treponema pallidum	Peptide in outer membrane	Surface protein (fibronectin)	Mucosal epithelium	Syphilis
Mycoplasma	Membrane protein	Sialic acid	Respiratory epithelium	Pneumonia
Chlamydia	Unknown	Sialic acid	Conjunctival or urethral epithelium	Conjunctivitis or urethritis

INVASION

The invasion of a host by a pathogen may be aided by the production of bacterial extracellular substances which act against the host by breaking down primary or secondary defenses of the body. Medical microbiologists refer to these substances as **invasins**. Most invasins are proteins (enzymes) that act locally to damage host cells and/or have the immediate effect of facilitating the growth and spread of the pathogen. The damage to the host as a result of this invasive activity may become part of the pathology of an infectious disease.

The extracellular proteins produced by bacteria which promote their invasion are not clearly distinguished from some extracellular protein toxins ("exotoxins") which also damage the host. Invasins usually act at a short range (in the immediate vicinity of bacterial growth) and may not actually kill cells as part of their range of activity; exotoxins are often cytotoxic and may act at remote sites (removed from the site of bacterial growth). Also, exotoxins typically are more specific and more potent in their activity than invasins. Even so, some classic exotoxins (e.g. diphtheria toxin, anthrax toxin) may play some role in colonization or invasion in the early stages of an infection, and some invasins (e.g. staphylococcal leukocidin) have a relatively specific cytopathic effect.

A Survey of Bacterial Invasins

Spreading Factors

"Spreading Factors" is a descriptive term for a family of bacterial enzymes that affect the physical properties of tissue matrices and intercellular spaces, thereby promoting the spread of the pathogen.

Hyaluronidase, is the original spreading factor. It is produced by streptococci, staphylococci, and clostridia. The enzyme attacks the interstitial cement ("ground substance") of connective tissue by depolymerizing hyaluronic acid.

Collagenase is produced by *Clostridium histolyticum* and *Clostridium perfringens*. It breaks down collagen, the framework of muscles, which facilitates gas gangrene due to these organisms.

Neuraminidase is produced by intestinal pathogens such as *Vibrio cholerae* and *Shigella*

dysenteriae. It degrades neuraminic acid (also called sialic acid), an intercellular cement of the epithelial cells of the intestinal mucosa.

Streptokinase and **staphylokinase** are produced by streptococci and staphylococci, respectively. Kinase enzymes convert inactive plasminogen to plasmin which digests fibrin and prevents clotting of the blood. The relative absence of fibrin in spreading bacterial lesions allows more rapid diffusion of the infectious bacteria.

Enzymes that Cause Hemolysis and/or Leucolysis

These enzymes usually act on the animal cell membrane by insertion into the membrane (forming a pore that results in cell lysis), or by enzymatic attack on phospholipids, which destabilizes the membrane. They may act as **lecithinases** or **phospholipases**, and if they lyse red blood cells they are sometimes called **hemolysins**. Leukocidins, produced by staphylococci and **streptolysin** produced by streptococci specifically lyse phagocytes and their granules. These latter two enzymes are also considered to be bacterial exotoxins.

Phospholipases, produced by *Clostridium perfringens* (i.e., alpha toxin), hydrolyze phospholipids in cell membranes by removal of polar head groups.

Lecithinases, also produced by *Clostridium perfringens*, destroy lecithin (phosphatidylcholine) in cell membranes.

Hemolysins, notably produced by staphylococci (i.e., alpha toxin), streptococci (i.e., streptolysin) and various clostridia, may be channel-forming proteins or phospholipases or lecithinases that destroy red blood cells and other cells (i.e., phagocytes) by lysis.
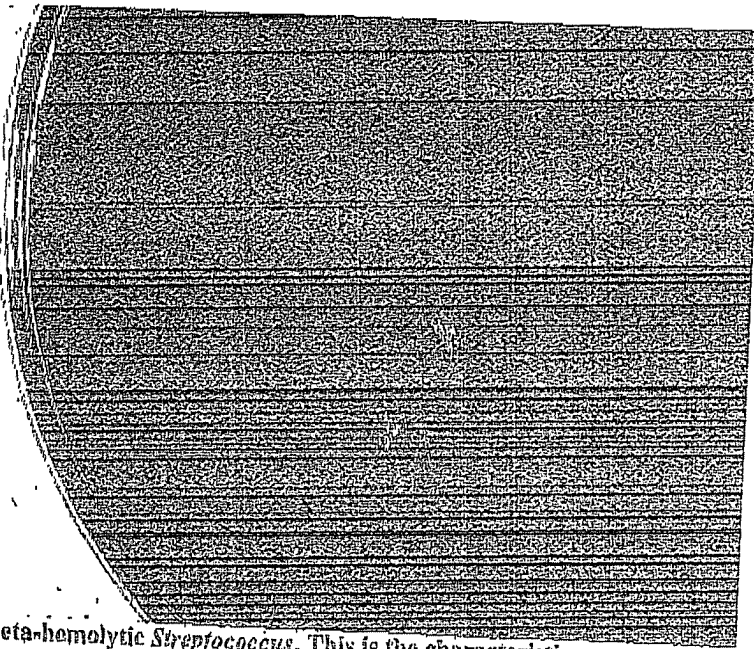
Beta-hemolytic *Streptococcus*. This is the characteristic appearance of a blood agar plate culture of the bacterium. Note the translucency around the bacterial colonies, representing hemolysis of the red cells in the culture medium due to production of a diffusible hemolysin (streptolysin).

Staphylococcal coagulase

Coagulase, formed by *Staphylococcus aureus*, is a cell-associated and diffusible enzyme that converts fibrinogen to fibrin which causes clotting. Coagulase activity is almost always associated with pathogenic *S. aureus* and almost never associated with nonpathogenic *S. epidermidis*, which has

led to much speculation as to its role as a determinant of virulence. Possibly, cell bound coagulase could provide an antigenic disguise if it clotted fibrin on the cell surface. Or a staphylococcal lesion encased in fibrin (e.g. a boil or pimple) could make the bacterial cells resistant to phagocytes or tissue bactericides or even drugs which might be unable to diffuse to their bacterial target.

Extracellular Digestive Enzymes

Heterotrophic bacteria, in general, produce a wide variety of extracellular enzymes including proteases, lipases, glycohydrolases, nucleases, etc., which are not clearly shown to have a direct role in invasion or pathogenesis. These enzymes presumably have other functions related to bacterial nutrition or metabolism, but may aid in invasion either directly or indirectly.

Toxins With Short-Range Effects Related to Invasion

Bacterial protein toxins which have adenylate cyclase activity are thought to have immediate effects on host cells that promote bacterial invasion. One component of the anthrax toxin (EF or Edema Factor) is an adenylate cyclase that acts on nearby cells to cause increased levels of cyclic AMP and disruption of cell permeability. One of the toxins of *Bordetella pertussis*, the agent of whooping cough, has a similar effect. These toxins may contribute to invasion through their effects on macrophages or lymphocytes in the vicinity which are playing an essential role to contain the infection. For example, since they use ATP as a substrate, they may deplete phagocyte reserves of energy needed for ingestion. Edema is seen as a pathology because the increase in cAMP in affected cells disrupts equilibrium.
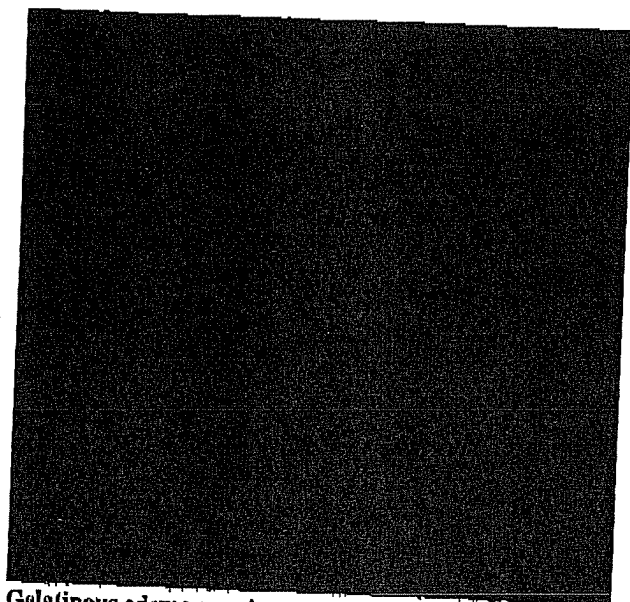
Gelatinous edema seen in a cutaneous anthrax lesion. CDC.

The following table summarizes the activities of many bacterial proteins that are noted for their contribution to bacterial invasion of tissues.

TABLE 3. SOME EXTRACELLULAR BACTERIAL PROTEINS THAT ARE CONSIDERED INVASINS

Invasin	Bacteria Involved	Activity
Hyaluronidase	Streptococci, staphylococci and clostridia	Degrades hyaluronic of connective tissue

Collagenase	Clostridium species	Dissolves collagen framework of muscles
Neuraminidase	Vibrio cholerae and Shigella dysenteriae	Degrades neuraminic acid of intestinal mucosa
Coagulase	Staphylococcus aureus	Converts fibrinogen to fibrin which causes clotting
Kinases	Staphylococci and streptococci	Converts plasminogen to plasmin which digests fibrin
Leukocidin	Staphylococcus aureus	Disrupts neutrophil membranes and causes discharge of lysosomal granules
Streptolysin	Streptococcus pyogenes	Repels phagocytes and disrupts phagocyte membrane and causes discharge of lysosomal granules
Hemolysins	Streptococci, staphylococci and clostridia	Phospholipases or lecithinases that destroy red blood cells (and other cells) by lysis
Lecithinases	Clostridium perfringens	Destroy lecithin in cell membranes
Phospholipases	Clostridium perfringens	Destroy phospholipids in cell membrane
Anthrax EF	Bacillus anthracis	One component (EF) is an adenylate cyclase which causes increased levels of intracellular cyclic AMP
Pertussis AC	Bordetella pertussis	One toxin component is an adenylate cyclase that acts locally producing an increase in intracellular cyclic AMP

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